Little association between birthweight and health of pre-weaned dairy calves

Corresponding author:
Ian D. Glover
West Ridge Veterinary Practice, 5 Chapple Road, Witheridge, Tiverton, Devon EX16 8AS, UK
idglover@hotmail.com
+44 (0) 1884 860236

Co-author
David C. Barrett
Bristol Veterinary School, University of Bristol, Langford House, Langford, Bristol BS40 5DU, UK

Co-author
Kristen K. Reyher
Bristol Veterinary School, University of Bristol, Langford House, Langford, Bristol BS40 5DU, UK

Word count: 3618

Keywords: Dairy cattle, Calves, Respiratory disease, Neonatal disease, diarrhoea

Abstract

Intrauterine growth retardation (IUGR) may result in reduced birthweight and detrimental physiological alterations in neonates. This prospective cohort study was designed to assess if there exists an association between birthweight of dairy calves and incidence of bovine respiratory disease (BRD), neonatal calf diarrhoea (NCD) or mortality during the pre-weaning period. Calves (n=476) on 3 farms in South West England were weighed at birth. Farmers kept records of treatments for NCD and BRD and calves were assessed weekly using clinical scoring systems (Wisconsin Calf Health Scores, California Calf Health Scores and Faeces Scores). Missing data were present in several variables. Multiple imputation coupled with generalised estimating equations (MI-GEE analysis) was employed to analyse associations between several calf factors, including birthweight, and probability of a case of BRD or NCD. Associations between calf factors and mortality were assessed using multiple logistic regression. Associations between birthweight and disease incidence were scarce. Birthweight was associated with odds of a positive Faeces Score on one farm only in the MI-GEE analysis (O.R. 1.03, 95% C.I. 1.0005 – 1.05, P=0.046). Birthweight was not associated with probability of mortality. This research suggests that birthweight, and therefore IUGR, is not associated with health of pre-weaned dairy calves.

1. Introduction

Pre-weaned dairy calf morbidity and mortality remains high. A UK study found 3.6 per cent mortality between 24 hours and 28 days, and 3.6 per cent between one and 6 months old [1]. Pre-weaning mortality ranged from 7.8 to 10.8 per cent in the USA [2]. Neonatal calf diarrhoea (NCD) and bovine respiratory disease (BRD) are predominant diseases [3] and, excepting stillbirth, the most common cause of mortality [4]. Heifer-rearing is a significant investment and disease reduces efficiency. The cost of rearing each heifer to calving has been found to be €1567 [5] and £1819 [6]. For a 100-cow herd, the annual rearing cost was US$32,344 [7]. Understanding factors which contribute to calfhood disease is desirable for welfare and economics reasons as well as environmentally sustainable and efficient food production. Birthweight (BW) is directed by genotype, but modified by gestation length (GL) [8,9] and uterine environment (UE) [10–12]. Intrauterine growth retardation (IUGR), whereby foetal development is modified by a suboptimal UE, is common amongst livestock [10] and causes much variation in BW [10,13]. Intrauterine growth retardation is mediated by nutrient limitation or alteration of placental size or function [10,12,14]. Causes include dam undernutrition [10,12,14,15], overnutrition [14,16] and nutrient-partitioning from gestation towards lactation in high-yielding cows or growth in immature heifers [10,12,17]. Negative energy balance and body condition score of the dam are associated with IUGR [11,12], as are disease and thermal stress [10,15]. Resource-sharing between foetuses in multiple pregnancies results in IUGR [10]. Intrauterine growth retardation affects organogenesis and immunity as well as overall foetal growth [18–21]. Consequences are dependent on retardation severity and on the stage of gestation at which it occurs [15]. Growth patterns of IUGR foetuses are therefore variable and dependent on the nature and timing of insults to which they are subjected.

Neonates which have been subjected to IUGR are at risk of various pathologies both in the short- and long-term. Documented consequences during the early postnatal period in livestock and humans include dysfunction of nervous, cardiovascular, digestive and endocrine organs; metabolic and hormonal abnormalities; immunodeficiency; and increased morbidity and mortality [10,15,22].

The conceptus may also adapt to a suboptimal UE through epigenetic modifications known as “foetal programming”, leading to permanent physiological changes with long-term consequences [10].

Few studies have examined IUGR and “foetal programming” in dairy cattle [12,14]. In light of the potential effects of IUGR on BW and health, this study aimed to investigate if there is an association between BW of dairy calves, and pre-weaning morbidity and mortality.

2. Materials and Methods

2.1 Data Collection

A convenience sample of Holstein and Holstein-Friesian calves on 3 farms in South West England was recruited. Farms were chosen because of their locality to the veterinary practice and their willingness to participate in the study. Table 1 shows details of herds and husbandry.

	Farm A	Farm B	Farm C
Herd size	490 cows	150 cows	285 cows
Breed	Holstein	Holstein-Friesian	Holstein-Swedish Red
Calving pattern	All year	Predominantly summer and autumn	Predominantly autumn
Colostrum provision	All calves receive 4 litres via oesophageal tube	Natural suckling, supplemented with oesophageal tube as deemed necessary	All calves receive 4 litres via oesophageal tube
Calving accommodation	Individual calving pens	Group calving straw yard	Individual calving pens
Calf accommodation	Housed and kept in groups of 5 animals from one day of age until weaning. Female and male calves kept in different sheds.	Housed in group pens of 5 animals until 10-14 days old, then housed in large group straw yards of 15-20 animals until weaning.	Individual calf hutches outside until 3 weeks of age. Group hutches outside thereafter until weaning.
Feeding	Twice daily 15% milk replacer fed up to a maximum of 6 litres of liquid per day. *Ad libitum* concentrate.	Twice daily whole milk up to 4 litres per day until 10-14 days old; thereafter 15% milk replacer fed by automatic feeder up to a maximum of 6 litres of liquid per day. *Ad libitum* concentrate containing 100 mg/kg decoquinate.	Twice daily 15% milk replacer fed up to a maximum of 6 litres of liquid per day. *Ad libitum* concentrate containing 100 mg/kg decoquinate.
Preventive treatments or vaccination	Heifer calves: halofuginone lactate (Halocur, MSD Animal Health, UK) and Intranasal PI3 and RSV vaccine (Rispoval RS+PI3 Intranasal, Zoetis, UK)	Vaccination of all late-gestation cows with combined rotavirus, coronavirus and *E. coli* K99 vaccine (Rotavec Corona, MSD Animal Health, UK)	All calves: halofuginone lactate (Halocur, MSD Animal Health, UK)
Period of calf recruitment	6th June 2014 - 3rd May 2015	6th July 2014 - 31st January 2015	17th September 2014 - 1st May 2015

Table 1: Details of herds and calf husbandry on the 3 farms.

Calves were eligible for recruitment if they were sired by a Holstein bull and were from singleton pregnancies. Calves were weighed by farm staff within 24 hours of birth using a calf weigh crate (Farms A and C; to the nearest kilogramme) or by placement of the calf in a bucket suspended from digital weigh scales (Farm B; to the nearest 100 grammes). Farmers recorded BW, sex and birth date. Farms were visited weekly by the first author or, rarely, another veterinarian. At each visit, calves born since the previous visit were blood sampled into anticoagulant-free blood tubes. Samples clotted at ambient temperature, and serum was decanted and centrifuged at 890g for 10 minutes. Serum total protein (STP) was estimated with a temperature-compensating optical refractometer, in line with normal practice protocols for managing herd health. Blood sampling was performed with approval from the Royal College of Veterinary Surgeons Ethics Committee.

At each visit all pre-weaned calves were assessed (Table 2) for BRD using the California Calf Health Score (CalCHS) [23] and the Wisconsin Calf Health Score (WisCHS) [4], and for NCD using a Faeces Score (FS) [4]. Farmers kept written records of treatments for BRD or NCD. The visiting veterinarian notified farmers of any calves showing overt signs of BRD (specifically calves with 2 or more of the following: pyrexia, dyspnoea or spontaneous coughing) or calves with a FS of at least 2. These overt clinical signs were chosen in order to emulate diagnosis based on diagnostic criteria commonly used by farm personnel, so as not to bias treatment data. Repeat diagnoses by health scoring or repeat treatments for the same disease were counted as a new incident if they were at least 7 days after the previous diagnosis or treatment. Dam parity was obtained from milk records and GL was calculated using farm records of service dates.

Wisconsin Calf Health Score (WisCHS) and California Calf Health Score (CalCHS)

Category	Observation	Score assigned	
		Wisconsin Calf Health Score†	California Calf Health Score‡
Nasal discharge	Normal serous discharge	0	0
	Small amount of unilateral cloudy discharge	1	4
	Bilateral, cloudy or excessive mucus discharge	2	4
	Copious bilateral mucopurulent discharge	3	4
Ocular discharge	Normal	0	0
	Small amount of ocular discharge	1	2
	Moderate amount of bilateral discharge	2	2
	Heavy ocular discharge	3	2
Rectal temperature °F (°C)	<100.9 (<38.3)	0	0
	101.0 – 101.9 (38.3 – 38.8)	1	0
	102.0 – 102.4 (38.9 – 39.1)	2	0
	102.5 – 102.9 (39.2 – 39.4)	2	2

	≥ 103.0 (≥39.5)	3	2
Ears and head	Normal	0	0
	Ear flick or head shake	1	0
	Slight unilateral droop	2	5
	Head tilt or bilateral droop	3	5
Cough*	None	0	0
	Single induced	1	0
	Repeated induced	2	0
	Occasional spontaneous	2	2
	Repeated spontaneous	3	2
Respiration§	Normal		0
	Abnormal		2

Faeces Score

Category	Observation	Score Assigned
Faeces∞	Normal	0
	Semi-formed, pasty	1
	Loose, but stays on top of bedding	2
	Watery, sifts through bedding	3

Table 2: Description of calf health scoring systems; Wisconsin Calf Health Score [4], California Calf Health Score [23] and Faeces Score [4].

* For the Wisconsin and California Calf Health Scores, coughing is induced by gently pinching the trachea.

† The Wisconsin Calf Health Score is the sum of the scores for rectal temperature, cough and nasal discharge, plus the score for ocular discharge or ears and head, whichever is greater. A positive score (i.e. a diagnosis of bovine respiratory disease, BRD) is a score greater or equal to 5 when at least 2 individual categories have a score of at least 2. http://www.vetmed.wisc.edu/dms/fapm/fapmtools/8calf/calf_health_scoring_chart.pdf

‡ The California Calf Health Score is the sum of the scores for each category. A positive score (i.e. a diagnosis of BRD) is a score greater or equal to 5 [23].

§ The Wisconsin Calf Health Score does not include assessment of respiration.

∞ A Faeces Score of greater or equal to 2 is considered abnormal.

2.2 Data Exploration

Data consisted of independent baseline variables and longitudinal, dependent health-outcome variables. Continuous baseline variables were birthweight (BW), gestation length (GL) and serum total protein (STP). Categorical baseline variables were SEX, SEASON (of birth) and FARM. Few older cows were present in the dataset, so PARITY (of the dam) was treated as an ordinal variable (1,2,3 or 4+). Longitudinal dependent variables were organised by week of life (WOL), with the aim of allocating one health score to each calf for each WOL. If a calf had greater than one health score for any WOL, the earlier of the 2 scores was deleted from the dataset. Therefore, for each WOL, each calf had data consisting of a positive or negative status for the following health outcomes: WisCHS, CalCHS, FS, farmer-diagnosis of BRD (fBRD) and farmer-diagnosis of NCD (fNCD).

Missing data within variables were quantified and explained in terms of their relationship with other variables. Data were considered missing at random (MAR) if missingness was associated with observed variables; missing completely at random (MCAR) if missingness was not associated with any variables; missing not at random (MNAR) if missingness was associated with unobserved (missing) variables [24]. Intermittent missingness within longitudinal data were instances where a health outcome was missing for a particular WOL and a health outcome was present in the dataset in a subsequent WOL for that calf. Monotone missingness (due to dropout) was missing health outcome data where all health outcome data were missing in subsequent WOLs for that calf.

2.3 Statistical Analysis

Multiple imputation [25] followed by generalised estimating equations (MI-GEE analysis) [26] were used for analysis. Data were stored and processed in Access and Excel. Statistical analysis was performed in R version 3.4.1 [27].

Sample size calculations were performed retrospectively using G*Power [28], based on the ability to detect a difference in probability of a positive diagnosis of disease of 0.1 (from 0.3 to 0.4) at 1 standard deviation from the mean BW.

2.3.1 Multiple Imputation

Baseline and longitudinal variables were imputed using the R package Amelia II [29]. Longitudinal (health outcome) data were imputed for all calves up to and including WOL 10. Prevalence of disease was expected to vary with WOL. For example, NCD incidence was likely higher during the first 2 weeks of life than during subsequent WOLs. Incorporation of the second-order polynomial of time into the imputation process allowed disease prevalence to vary with calf age, and also allowed the pattern of change of disease prevalence over time to vary between farms. Thirty datasets were imputed.

Validity of multiple imputation was assessed by visual comparison of the distribution of observed and imputed data.

2.3.2 Generalised Estimating Equations

Correlation was expected between health outcomes during different WOLs for any given calf. Generalised estimating equations with a logit link were constructed using the R package Zelig [30], using Rubin's rule for combination of multiply imputed datasets. Calf identification indicated clusters. Models were constructed for each dependent variable: WisCHS, CalCHS, FS, fBRD and fNCD. Covariance structure was chosen by comparing the quasi-likelihood under the independence model criterion (QIC) for initial models created using differing covariance structures. Exchangeable covariance structures were used for the WisCHS, CalCHS and fNCD models, whilst autoregressive covariance structures were used for the FS and fBRD models. Initial models were created using all independent variables including WOL, plus quadratic and cubic transformations of BW, to allow for non-linear associations between BW and dependent variables. Backwards model selection was performed according to the change in QIC, until the most parsimonious model was found. Variables were investigated for confounding and retained if their removal resulted in greater than 30 per cent change in coefficients of variables with $P<0.05$. Plausible 2-way interactions between each permutation of covariate pairs were tested by introducing them to the models, and interactions were retained if $P<0.05$.

2.3.3 Analysis of Calf Mortality

A second, non-imputed dataset was constructed including only calves that were not sold. The same predictor variables were used, and the binary dependent variable MORTALITY was defined as death or euthanasia prior to weaning. One multivariable logistic regression model for MORTALITY was constructed using the second dataset. Significance was assessed using the Z-value. Variables with $P<0.25$ in univariable analysis were included in initial models [31]. FARM and BIRTHWEIGHT were forced into models, to examine the association of BIRTHWEIGHT with the dependent variable and to account for clustering within farms. Covariates were eliminated in a backwards stepwise fashion until only terms with $P<0.05$, plus BIRTHWEIGHT and FARM, remained. As above, variables were investigated for confounding and retained if their removal resulted in greater than 30 per cent change in coefficients of variables with $P<0.05$. Quadratic and cubic transformations of BIRTHWEIGHT were offered to the model to allow for non-linear associations.

All 2-way interactions were added in turn to the model and were retained if biologically plausible and if $P<0.05$. Goodness of fit was assessed using the Hosmer-Lemeshow Goodness of Fit test, following comparison of number of covariate patterns with number of subjects. Predictive ability of the model was assessed with receiver-operating characteristic analysis. Plots of delta-deviance, delta Pearson Chi Square and delta-beta were examined. The model was rebuilt following removal of influential data points and the new model was accepted if outliers were considered to be unduly influencing the conclusions drawn.

3. Results

3.1 Descriptive Statistics

476 calves were recruited during the study period. The median interval between consecutive health scores for any calf was 7 days and the percentage of intervals that were less than or equal to 9 days was 93. The median number of health scores per calf was 4 for males and 10 for females, due to a greater number of male calves dying, being sold or euthanized. Age at weaning was variable (median

76.0 d, min 33.0 d, max 110.0 d). Table 3 describes the distribution of variables prior to multiple imputation.

		Farm A	Farm B	Farm C	Total
Number of calves		341	55	80	476
Sex	Male	175	20	39	234
	Female	166	35	41	242
Birth weight (Kg)	Median	42.0	42.1	39.0	42.0
	Interquartile Range	38.0 – 46.0	38.0 – 44.5	37.0 – 42.0	38.0 – 45.0
	Min	26.0	32.3	29.0	26.0
	Max	62.0	49.7	51.0	62.0
Serum total protein (g/dl)	Median	5.2	5.8	5.6	5.4
	Interquartile Range	4.8 – 5.6	5.2 – 6.7	5.2 – 6.2	4.9 – 5.8
	Min	3.1	4.0	3.7	3.1
	Max	7.2	8.4	7.8	8.4
Season of birth (number of calves)	Spring	72	0	20	92
	Summer	69	7	1	77
	Autumn	105	34	30	169
	Winter	95	14	29	138
Parity of dam (number of calves)	1	115	7	27	149
	2	86	27	12	125
	3	63	8	15	86
	≥4	65	13	24	102
Percentage of calves with FPT*		49	26	23	42

Table 3: Characteristics of calves in the dataset prior to multiple imputation.

*FPT = Failure of passive transfer, defined by serum total protein <5.2 g/dl

Table 4 describes disease incidence on the 3 farms during the study period.

	Farm A	Farm B	Farm C	Total
Number of Calf Health Scores[*]	2032	357	438	2827
Percentage of calves receiving at least one positive Wisconsin Calf Health Score[†]	74.9	64.9	33.9	67.4
Percentage of calves receiving at least one positive California Calf Health Score[†]	69.1	51.4	37.1	62.3
Percentage of calves receiving at least one positive Faeces Score[†]	53.6	51.4	46.8	52.3
Percentage of calves receiving at least one treatment for bovine respiratory disease (BRD)[‡]	58.8	40.5	9.7	49.2
Percentage of calves receiving at least one treatment for neonatal calf diarrhoea (NCD)[‡]	12.4	21.6	1.6	11.5

Disease incidence (cases/calf/week)§	Positive Wisconsin Score	0.3	0.1	0.07	0.2
	Positive California Score	0.2	0.1	0.1	0.2
	Positive Faecal Score	0.1	0.07	0.06	0.09
	BRD treatment	0.1	0.05	0.01	0.09
	NCD treatment	0.02	0.02	0.00	0.02
Mortality (%)		14.4	5.4	1.6	11.5
Euthanized (%)		3.0	3.0	0.0	3.0
Sold Prior to Weaning (%)		37.0	0.0	44.0	35.0
Weaned (%)		45.6	91.6	54.4	50.5

Table 4: Percentage of calves with at least one disease incident, overall disease incidence and fate of calves along with detailed information on each of the 3 farms.

*Number of health scores in the dataset for each farm and overall

†Percentage of calves (on each farm and overall) receiving at least one positive Wisconsin Calf Health Score, California Calf Health Score or Faeces Score prior to exit from the study through sale, death, euthanasia or weaning. A positive Wisconsin Calf Health Score or positive California Calf Health Score represents a diagnosis of bovine respiratory disease (BRD). A positive Faeces Score represents a diagnosis of neonatal calf diarrhoea (NCD).

‡Percentage of calves (on each farm and overall), which received at least one treatment for BRD or NCD.

§Incidence of disease according to Calf Health Scores and farm records of disease treatment. Incidence was calculated by dividing the total number of disease or treatment incidents by number of calf-weeks. Positive Calf Health Scores or disease treatments were counted as disease incidents if there had been no previous diagnosis of the same disease in the same calf within 7 days.

A total sample size of 290 was required to detect a difference in probability of a positive diagnosis of BRD of 0.1 at one standard deviation from the mean BW.

3.2 Missing data

The proportion of missing data for each variable prior to multiple imputation is described in Figure 1. Missingness within the longitudinal health outcome variables increased as WOL increased due to monotone dropout. For the baseline variables, missingness was greatest within the GL variable, at 29.2%. Data were subject to missingness within all but the following variables: SEX, FARM and SEASON. Reasons for missingness were errors in collecting or recording data (intermittent missingness) and dropout of calves prior to weaning due to death, euthanasia or sale (monotone missingness). Intermittent missingness was mainly considered to be missing completely at random (MCAR) as failure to collect or record data was due to human error and was not conceivably influenced by any of the observed data. However, in the case of the GL variable, missingness was observed predominantly in calves from primiparous dams on Farm A. This was due to the use of natural service in heifers, which precluded the recording of service dates and thus calculation of GL. Thus missing GL data were considered to be missing at random (MAR). Birthweight was missing for several calves born during winter months, and this was due to a reluctance by farmers to weigh calves over the Christmas period. Missingness in the BW variable was therefore considered to be MAR. Most missingness within the STP variable was in calves born during autumn. This was due to some blood samples being lost during a short period in Autumn 2014. STP missingness was therefore MAR. Monotone missingness of the health outcome data due to dropout was MAR as missingness may have been dependent on observed data (for example mortality of calves associated with low STP), but was not conceivably dependent on missing data. Amongst calves with missing health outcome data, males were overrepresented, especially on Farm A, reflecting the sale of male calves prior to weaning. Table 5 describes the distribution of variables for calves with complete data and calves with data missing within individual variables.

Variable with missingness											
		None (Complete data)	BW	STP	Gestation Length	Parity Category	WisCHS	CalCHS	Faeces Score	fBRD	fNCD
Median BW (IQR)		42.4 (39.0 – 46.0)		42.0 (38.1 – 44.9)	38 (36 – 43)	44.0 (43.0 – 48.0)	42.0 (38.0 – 45.1)	42 (38.0-45.1)	42 (38 – 45.1)	42 (38 – 46)	42 (38 – 46)
Median STP (IQR)		5.3 (4.9 – 5.8)	5.4 (5.1 – 5.8)		5.1 (4.7 – 5.6)	NA	5.2 (4.9-5.8)	5.2 (4.9 – 5.8)	5.2 (4.9 – 5.8)	5.2 (4.8 – 5.7)	5.2 (4.8 – 5.7)
SEX (Number of calves)	Male	139	17	29	68	9	227	227	227	217	217
	Female	144	16	27	71	5	169	169	169	116	116
SEASON (Number of calves)	Spring	55	2	2	36	1	80	80	80	72	72
	Summer	64	2	0	11	0	63	63	63	57	57
	Autumn	98	3	41	42	8	144	144	144	115	115
	Winter	66	26	13	50	5	109	109	109	89	89
Median GL (IQR)		280 (277 – 283)	283 (276.5 – 285.5)	278 (275.8 – 282.0)		NA	280 (277 – 283)	280 (277 – 283)	280 (277 – 283)	280 (277 – 283)	280 (277 – 283)
Parity Category (Number of calves)	1	25	1	13	116		123	123	123	106	106
	2	98	6	16	5		103	103	103	84	84
	3	74	3	7	2		75	75	75	63	63
	4+	86	9	6	2		81	81	81	66	66
Farm	A	181	26	29	136	12	281	281	281	249	249
	B	39	0	16	0	0	46	46	46	23	23
	C	63	7	11	3	2	69	69	69	61	61

Table 5: Distribution of variables for calves with no missing data or missing data in each of the covariates.

NA=Missingness affecting 2 variables simultaneously (e.g. all calves with missing parity category data also had missing gestation length data)

3.3 Multiple Imputation

Uneventful convergence of imputation algorithms was confirmed by the Amelia II package. Visual examination of plots of non-imputed and imputed data confirmed that distributions of imputed data were within the lower and upper limits of values for non-imputed data. Time-series-cross-sectional plots confirmed that prevalence of disease varied with WOL in imputed data.

3.4 Generalised Estimating Equations

A significant association between BW and the dependent variable was found in only the Faeces Score model. In this model there was a significant interaction between BW and Farm such that increasing BW was associated with an increase in the odds of a positive Faeces Score on Farm A only (O.R. 1.03, 95% C.I. 1.0005 – 1.05, P=0.046). BW was not associated with any other health outcomes. Increasing STP was associated with lower odds of a positive CalCHS (O.R. 0.82, 95% C.I. 0.72 – 0.93, P=0.002) and there was a trend towards an association between STP and odds of a positive WisCHS (O.R. 0.87, C.I. 0.76 – 1.00, P=0.05). STP was not associated with odds of any other outcomes. Calves born during Spring had higher odds of fBRD (O.R. 1.51, 95% C.I. 1.07 – 2.14, P=0.02) compared to calves born during other seasons. There was also a trend towards an association between Season of birth and odds of a positive WisCHS, with calves at higher risk during Winter and Spring (O.R. 1.25, 95% C.I. 0.98 – 1.58, P=0.07). Gestation length and parity were not associated with any of the outcomes. Calves on Farm A had higher odds of disease than calves on Farms B and C in all 3 BRD models (WisCHS, CalCHS and fBRD). Sex was associated with the outcome in several models. For 2 of the BRD models male calves had significantly higher odds of disease on all farms (WisCHS O.R. 1.46, 95% C.I. 1.21 – 1.75, P=0.00007; fBRD O.R. 1.35, 95% C.I. 1.06 – 1.72, P=0.02). A significant interaction emerged between Sex and Farm in the CalCHS, Faeces Score and FNCD models such that male calves had higher odds of these disease outcomes on Farm A only. Week of life was often associated with odds of disease outcomes (data not shown). For example, odds of a positive WisCHS or CalCHS showed a quadratic association with WOL, with highest odds in WOL 3 for WisCHS and fBRD, and in WOL 5 for CalCHS. For Faeces Scores and fNCD, odds of a positive diagnosis were highest in WOL 1, thereafter declining in subsequent weeks. Prevalence of disease in different WOLs is shown in Figure 2. No significant interactions were found between WOL and any other variable.

3.5 Analysis of Mortality

In order to preserve sample size, calves with missing GL were retained in the dataset and the GL variable was not included in any models. Following deletion from the dataset of calves with missing data in the remaining baseline variables, 390 calves remained. Following deletion of calves that were sold, 244 remained. Of all covariates in the model, STP alone was associated with odds of mortality (O.R. 0.39, 95% C.I. 0.158 – 0.940, P=0.036). No significant interactions between covariates were found.

4. Discussion

In this study, BW was rarely associated with any health outcomes. In the GEE models BW was associated only with odds of a positive Faeces Score on one farm. Type-1 error may explain this single association. However, lack of association in GEE models between BW and Faeces Scores on the other 2 farms or between BW and health outcomes in all other models is surprising in light of evidence that IUGR may result in organ dysfunction [10]. It is possible that IUGR is associated with increased risk of disease in later life, as in humans [32]. Calves in this study were only observed until weaning. Dystocial calves are more likely to suffer morbidity [33,34] and mortality [33–35] subsequent to the perinatal period. Perhaps prevalence of dystocia was highest on Farm A due to greater BW or to some other unmeasured factor. This could explain the association of higher birthweight with increased odds of positive Faeces Scores on this farm. However, the linear association in this model suggests medium BW calves on Farm A had higher odds of diarrhoea than low BW calves. This is unlikely to be due to dystocia as predominantly calves with high birthweights would be expected to have experienced calving difficulty. Calves on all 3 farms were not fed according to size, as all calves in any age group were fed the same, so smaller calves were possibly on a comparatively high plane of nutrition, resulting in increased resilience to disease. Farmers were not blinded to BW so husbandry of smaller calves may have been improved consciously or subconsciously on Farm A only.

The findings of this study contrast with previous work which has found associations between low BW and disease or mortality. Windeyer and others [36] found low BW heifer calves have higher odds of NCD. Although least squares mean (LSM) BW (38 kg) was slightly lower than mean female BW in the current study, BW distribution was not described. A study by Corah and others [37] found low BW beef calves from nutrient-restricted dams had higher NCD incidence. Again, BW distribution was not described, but LSM BW of the lightest category was 26.7 kg, only slightly greater than the lowest BW in the current study. It is difficult to draw BW comparisons due to the differing genetics of calves across studies, but perhaps those 2 studies [36,37] included calves of lower BW and more subjected to IUGR than those in the current study.

Other researchers [38] found both low and high BW Holstein calves on 2 Californian farms succumbed to NCD sooner than medium BW calves during winter. Birthweight ranged from 29 to 68 kg (mean 41.5 kg), similar to the current study, but with greater range of BW. The authors speculated that small calves experienced thermal stress during winter, and large calves suffered dystocia, causing earlier NCD onset. Minimum Californian winter temperatures were unlikely to be substantially lower than South West England, and the smallest calves in the study were larger than the smallest calves in the current study. Calves in the present study were born during all seasons, and no significant interactions between season and BW were found. Perhaps if time-to-onset of NCD had been measured in the current study an association would have been found with low BW.

Varying associations have been found between BW and mortality of calves over 48 hours old. McCorquodale and others [39] found low birthweight Holstein heifer calves (under 39 kg) were more likely to die before 90-120 days of age. Another large scale study by Moore and others [40] of Holstein bull calves found that low BW (under 48 kg) was associated with increased mortality prior to 3 weeks old[40]. Henderson and others [41] found that both low (under 37 kg) and high (over 42 kg) BW female Holstein calves were more likely to die prior to first calving.

Henderson and others included calves with lower BW (minimum 22 kg) than the current study. If the present study had included calves with such low BW, an association between BW and mortality may have been evident. However, the definitions of low BW made by McCorquodale and others and Moore and others were high compared to the current study, and yet in those studies lower BW was associated with mortality. Calves in the present study were only observed until weaning, whilst Henderson and others studied animals until first calving (and most mortalities occurred post-weaning) and McCorquodale and others followed animals until 90-120 days old. It would appear that on the whole previous studies have found an association between low BW and poor outcomes for calves, in contrast to the present study. Again, perhaps BW is associated less with disease incidence in the pre-weaned period than in later life.

Gestation length is an important confounder in that it is associated with birthweight and may be associated with increased risk of neonatal disease, for example through reduced intestinal absorption of immunoglobulins immediately following birth [42]. It is conceivable that some IUGR calves in this study had birthweights closer to the mean due to gestation lengths that were greater than average. As GL was not included as a predictor in the mortality model, a tendency to find no association between BW and mortality may have resulted. However, the study by Corah and others [37] found that induction of IUGR through feed restriction of late-gestation cows led to reduced calf birthweight and reduced gestation length, which does not support such speculation. In the studies [36,39–41,38] discussed above which found an association between birthweight and disease or mortality, gestation length of dams was not described, so it may be that the datasets included premature calves which were of low birthweight and more susceptible to disease. Future studies on the subject of IUGR would benefit from the measurement of gestation length.

The aim of this study was to investigate the association of BW, and indirectly of IUGR, with disease incidence. One factor, not measured in this study, which influences BW through mechanisms other than IUGR, is genetics [13,43]. The inclusion of some measure of genetic effect on BW in the regression models, for example sire identity or percentage Holstein genotype of the dam, may have improved the statistical modelling.

5. Conclusions

This paper suggests that low birthweight, and thus IUGR, is not associated with susceptibility to respiratory or enteric infections in dairy calves during the pre-weaning period.

6. Conflict of interest statement

None of the authors has any association with persons or organisations which could inappropriately influence the contents of this paper.

7. Acknowledgements

The authors would like to express thanks to participating colleagues and clients of The Vale Veterinary Group, Devon, U.K. for their kind assistance during this project. Thanks also go to MSD Animal Health for funding the study and in particular Paul Williams MRCVS for his help with preparation of this paper. Much gratitude is also due to the Farm Animal Group at Bristol Veterinary School for their suggestions and assistance in reviewing this work, and to Dr. Bobby Stuijfzand for guidance on statistical methods.

8. Literature Cited

1. Brickell JS, McGowan MM, Pfeiffer DU, Wathes DC. Mortality in Holstein-Friesian calves and replacement heifers, in relation to body weight and IGF-I concentration, on 19 farms in England. *Animal*. 2009;**3**:1175.

2. Gorden PJ, Plummer P. Control, management, and prevention of bovine respiratory disease in dairy calves and cows. *Vet Clin North Am - Food Anim Pract*. 2010;**26**:243–59.

3. Johnson KF, Chancellor N, Burn CC, Wathes DC. Prospective cohort study to assess rates of contagious disease in pre-weaned UK dairy heifers: management practices, passive transfer of immunity and associated calf health. *Vet Rec Open*. 2017;**4**:e000226.

4. McGuirk SM. Disease management of dairy calves and heifers. *Vet Clin North Am - Food Anim Pract*. 2008;**24**:139–53.

5. Mohd Nor N, Steeneveld W, Mourits MCM, Hogeveen H. Estimating the costs of rearing young dairy cattle in the Netherlands using a simulation model that accounts for uncertainty related to diseases. *Prev Vet Med*. 2012;**106**:214–24.

6. DairyCo. An economic analysis of heifer rearing and breeding selection in Great Britain – an empirical analysis. Results of calf and heifer rearing survey. 2015. http://dairy.ahdb.org.uk/resources-library/research-development/health-welfare/an-economic-analysis-of-heifer-rearing-and-breeding-selection-in-great-britain-—-an-empirical-analysis/#.V4_RErgrLtQ (accessed 20th July 2016)

7. Tozer PR, Heinrichs AJ. What affects the costs of raising replacement dairy heifers: a multiple-component analysis. *J Dairy Sci*. 2001;**84**:1836–44.

8. Dhakal K, Maltecca C, Cassady JP, Baloche G, Williams CM, Washburn SP. Calf birth weight, gestation length, calving ease, and neonatal calf mortality in Holstein, Jersey, and crossbred cows in a pasture system. *J Dairy Sci*. 2013;**96**:690–8.

9. Kamal MM, Van Eetvelde M, Depreester E, Hostens M, Vandaele L, Opsomer G. Age at calving in heifers and level of milk production during gestation in cows are associated with the birth size of Holstein calves. *J Dairy Sci*. 2014;**97**:5448–58.

10. Wu G, Bazer FW, Wallace JM, Spencer TE. Board-invited review: Intrauterine growth retardation: Implications for the animal sciences. *J Anim Sci*. 2006;**84**:2316–37.

11. Banos G, Brotherstone S, Coffey MP. Prenatal maternal effects on body condition score, female fertility, and milk yield of dairy cows. *J Dairy Sci*. 2007;**90**:3490–9.

12. Bach A. Nourishing and managing the dam and postnatal calf for optimal lactation, reproduction and immunity. *J Anim Sci*. 2012;**90**:1835–45.

13. Swali A, Wathes DC. Influence of the dam and sire on size at birth and subsequent growth, milk production and fertility in dairy heifers. *Theriogenology*. 2006;**66**:1173–84.

14. Schoonmaker J, Eastridge M. Effect of maternal nutrition on calf health and growth. *Proc 22nd Tri-State Dairy Nutr Conf*. 2013;**26**:63–80.

15. Symonds ME, Sebert SP, Budge H. Nutritional regulation of fetal growth and implications for productive life in ruminants. *Animal*. 2010;**4**:1075–83.

16. Wallace JM, Luther JS, Milne JS, Aitken RP, Redmer DA, Reynolds LP, et al. Nutritional modulation of adolescent pregnancy outcome - a review. *Placenta*. 2006;**27**:61–8.

17. Green JC, Meyer JP, Williams AM, Newsom EM, Keisler DH, Lucy MC. Pregnancy development from day 28 to 42 of gestation in postpartum Holstein cows that were either milked (lactating) or not milked (not lactating) after calving. *Reproduction*. 2012;**143**:699–711.

18. Long NM, Vonnahme KA, Hess BW, Nathanielsz PW, Ford SP. Effects of early gestational undernutrition on fetal growth, organ development, and placentomal composition in the bovine. *J Anim Sci*. 2009;**87**:1950–9.

19. Caton JS, Vonnahme KA, Luther JS, Lardy GP. Nutritional management during gestation : impacts on lifelong performance. In: Proceedings of the 18th Annual Florida Ruminant Nutrition Symposium. 2007. p. 1–20.

20. Meyer AM, Reed JJ, Vonnahme KA, Soto-Navarro SA, Reynolds LP, Ford SP, et al. Effects of stage of gestation and nutrient restriction during early to mid-gestation on maternal and fetal visceral organ mass and indices of jejunal growth and vascularity in beef cows. *J Anim Sci*. 2010;**88**:2410–24.

21. Moore SE, Collinson AC, Tamba N'Gom P, Aspinall R, Prentice AM. Early immunological development and mortality from infectious disease in later life. *Proc Nutr Soc*. 2006;**65**:311–8.

22. Sharma D, Shastri S, Sharma P. Intrauterine Growth Restriction: Antenatal and Postnatal Aspects. *Clin Med Insights Pediatr*. 2016;**10**:CMPed.S40070.

23. Love WJ, Lehenbauer TW, Kass PH, Van Eenennaam AL, Aly SS. Development of a novel clinical scoring system for on-farm diagnosis of bovine respiratory disease in pre-weaned dairy calves. *PeerJ*. 2014;**2**:e238.

24. Sterne JAC, White IR, Carlin JB, Spratt M, Royston P, Kenward MG, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. *BMJ*. 2009;**338**:b2393–b2393.

25. Dohoo IR, Nielsen CR, Emanuelson U. Multiple imputation in veterinary epidemiological studies: A case study and simulation. *Prev Vet Med*. 2016;**129**:35–47.

26. Aloisio KM, College S, Swanson SA, Horton NJ. Analysis of partially observed clustered data using generalized estimating equations and multiple imputation. *Stata J*. 2014;**14**:863–83.

27. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2017. https://www.r-project.org.

28. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. *Behav Res Methods*. 2009;**41**:1149–60.

29. Honaker J, King G, Blackwell M. AMELIA II : A Program for Missing Data. *J Stat Softw*. 2011;**45**:1–47.

30. Choirat C, Honaker J, Imai K, King G, Lau O. Zelig: Everyone's Statistical Software. Version 5.1-3. 2017. http://zeligproject.org/.

31. Hosmer DW, Lemeshow S. Variable Selection. In: Applied Logistic Regression. 2nd ed. John Wiley & Sons, Inc.; 2000. p. 92–116.

32. Barker DJ. In utero programming of chronic disease. *Clin Sci*. 1998;**95**:115–28.

33. Lombard JE, Garry FB, Tomlinson SM, Garber LP. Impacts of dystocia on health and survival of dairy calves. *J Dairy Sci*. 2007;**90**:1751–60.

34. Barrier AC, Haskell MJ, Birch S, Bagnall A, Bell DJ, Dickinson J, et al. The impact of dystocia on dairy calf health, welfare, performance and survival. *Vet J*. 2013;**195**:86–90.

35. Wells SJ, Dargatz DA, Ott SL. Factors associated with mortality to 21 days of life in dairy heifers in the United States. *Prev Vet Med*. 1996;**29**:9–19.

36. Windeyer MC, Leslie KE, Godden SM, Hodgins DC, Lissemore KD, LeBlanc SJ. Factors associated with morbidity, mortality, and growth of dairy heifer calves up to 3 months of age. *Prev Vet Med*. 2014;**113**:231–40.

37. Corah LR, Dunn TG, Kaltenbach CC. Influence of prepartum nutrition on the reproductive performance of beef females and the performance of their progeny. *J Anim Sci*. 1975;**41**:819–24.

38. Paré J, Thurmond MC, Gardner I a., Picanso JP. Effect of birthweight, total protein, serum IgG and packed cell volume on risk of neonatal diarrhea in calves on two California dairies. *Can J Vet Res*. 1993;**57**:241–6.

39. McCorquodale CE, Sewalem A, Miglior F, Kelton D, Robinson A, Koeck A, et al. Short communication: analysis of health and survival in a population of Ontario Holstein heifer calves. *J Dairy Sci*. 2013;**96**:1880–5.

40. Moore DA, Sischo WM, Festa DM, Reynolds JP, Atwill ER, Holmberg CA. Influence of arrival weight, season and calf supplier on survival in Holstein beef calves on a calf ranch in California, USA. *Prev Vet Med*. 2002;**53**:103–15.

41. Henderson L, Miglior F, Sewalem A, Kelton D, Robinson A, Leslie KE. Estimation of genetic parameters for measures of calf survival in a population of Holstein heifer calves from a heifer-raising facility in New York State. *J Dairy Sci*. 2011;**94**:461–70.

42. Johnston NE, JA S. The effect of glucocorticoids and prematurity on absorption of colostral immunoglobulin in the calf. *Aust Vet J*. 1986;**6**:191–2.

43. Koçak S, Tekerli M, Özbeyaz C, Yüceer B. Environmental and genetic effects on birth weight and survival rate in Holstein Calves. *Turkish J Vet Anim Sci*. 2007;**31**:241–6.

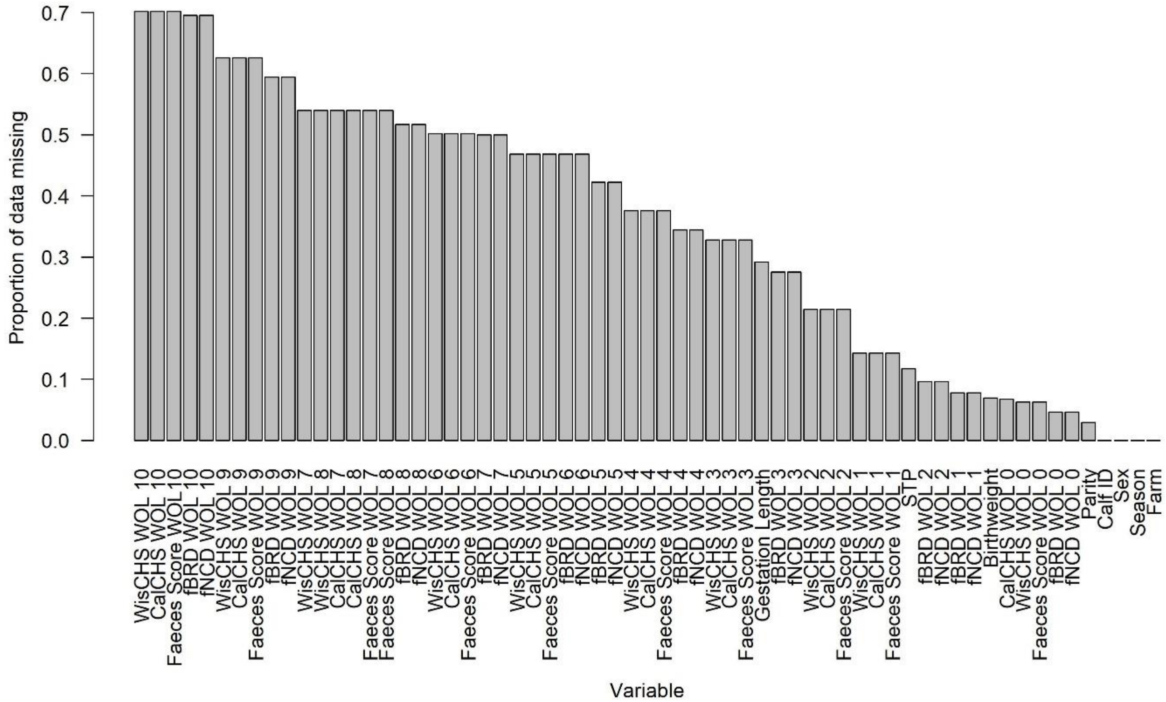

Figure 1: Proportion of subjects in the dataset for which data was missing in each variable. WisCHS=Wisconsin Calf Health Score, CalCHS=California Calf Health Score, fNCD=Farmer-recorded neonatal calf diarrhoea, fBRD=Farmer-recorded bovine respiratory disease, STP=serum total protein, WOL=Week of life (WOL 0 = 0 to 7 days of age, WOL 1 = 8 to 14 days of age etc.)

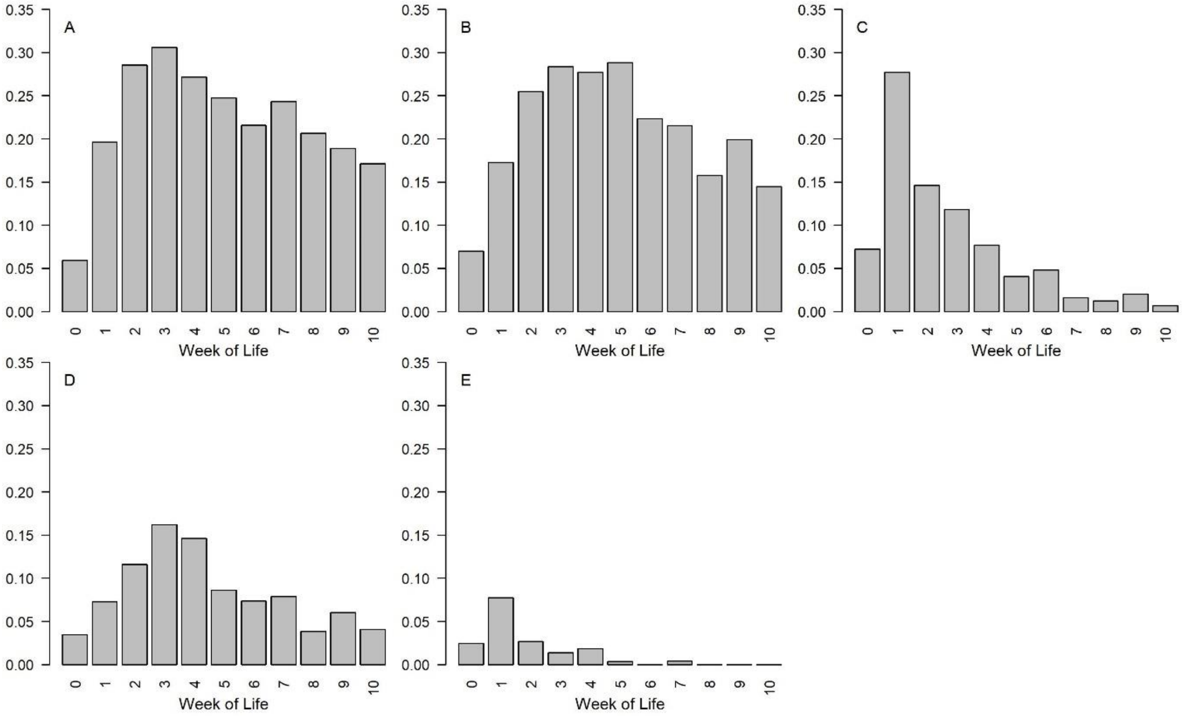

Figure 2: Proportion of pre-weaned calves diagnosed by different methods with disease in each week of life. A=Wisconsin Calf Health Score, B=California Calf Health Score, C=Faeces Score, D=Farmer-recorded bovine respiratory disease (BRD), E=Farmer-recorded neonatal calf diarrhoea (NCD).